Reproductive cycle, number of parities and faecal *Salmonella* spp. excretion in sows: a longitudinal study

Magistrali C.F.*[(1)], D'Avino N. [(1)], Ciuti F. [(1)], Cucco L. [(1)], Maresca C. [(1)], Paniccià M. [(1)], Scoccia E. [(1)], Tentellini M. [(1)], Pezzotti G. [(1)].

1 Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy

**corresponding author: c.magistrali@izsum.it*

Abstract

The aim of the study was to evaluate the fecal excretion of *Salmonella* spp. in sows with different number of parities, in distinct periods of the reproductive cycle. Two integrated farms in central Italy have been included in the study. The target populations were primaparous and multiparous (1-5 and more than 5 parities) sows, tested in different periods of the reproductive cycle: 14 days before partuition (pre-partum), 2-5 days (post-partum 1), 20 days after parturition (post-partum 2) and gestation (24-31 days post-partum). The subjects were considered positive if *Salmonella* was isolated from faeces. In addition, environmental samples were collected from farrowing rooms and gestation boxes, before the introduction of the sows. During the pre-partum phase one single sow resulted positive, three in post partum 1, six in post partum 2 and 27 in gestation. *S.* Munchen and *S.* Typhimurium were isolated from the sows on the first farm, while *S.* Choleraesuis and *S.* 4,5,12:i- were the two serotypes found on farm 2. The incidence density for *Salmonella* was 0,007 days for the group of primiparous sows, 0.005 days for the pluriparous (1-5) and 0.0001 days for the group with more than 5 parities. The cumulative incidence resulted 0.6 % (I.C.95%=0%-2%) in pre-partum, 2% (I.C.95%=0%-4%) in post partum 1, 4% (I.C.95%=1%-8%) post partum 2 and 27% (I.C.95%=18%-36%) in gestation. *Salmonella* was also isolated from the environmental samples collected in the farrowing rooms (5 out of 62; 8%) and the gestation boxes (28 out of 120; 23%); *S.* Munchen was isolated from the first farm while *Salmonella* 4,5,12:i- and *S.* Typhimurium var. Copenhagen were isolated on the second farm.
The incidence values demonstrate that young sows tend to get infected more rapidly than the older ones. Moreover, gestation represents a critical period for the excretion of *Salmonella*, probably related to environmental challenge.

Introduction

Salmonella still represents one of the major agents of foodborne diseases in humans; Salmonellosis, with its 151,995 cases registered in 2007 (EFSA 2009), is the second zoonosis after campylobacteriosis in Europe. Pork, after chicken and eggs, is considered one of the most relevant sources of infection; in a survey carried out in 2007, 1.1% of the fresh pork meat samples collected in Europe resulted positive (EFSA, 2009), while the occurrence of *Salmonella* in swine mesenteric lymph nodes at slaughter was 10.3% across Europe and 16.5% in Italy (EFSA 2008). In the perspective of a progressive reduction of salmonellosis in the European Union, several control measures were taken into consideration at different steps along the production chain, since a holistic approach is required to finally reduce the bacterial load in the final products. Numerous studies were carried out at different levels (pre-harvest, transport, slaughter, processing and distribution) in order to clarify the epidemiology of *Salmonella* infection and identify the most efficacious control measures in the practice of the production. With regards to pre-harvest, several transmission routes were described in pigs, where a significant role was demonstrated both for direct and indirect transmission (environmental contamination).In particular, the role of sows in the maintenance of the infection within the herd was outlined, although in these groups the prevalence is usually lower than in fattening pigs. This role is more relevant in farrow to finish farms, where cross contamination between groups (reproduction and fattening) seems more frequent (Nollet, 2005). Based on this epidemiological data, intervention strategies in the EU are increasingly focused on prevention at the top levels of the process, particularly in the reproduction phase, to prevent the risk of introducing

Salmonella by animals (EFSA, 2008). Previous works have outlined high serological prevalence of infection (from 93.8% to 100%) also in breeding pig herds in Italy (Merialdi, 2008).To better clarify the dynamics of infection in sows, a cohort study was carried out in two farrow to finish pig farms in central Italy, to evaluate the excretion rate of *Salmonellae* with faeces in relation to the stage of the reproductive cycle and the number of parities.

Materials and methods

The study was carried out from December 2006 to November 2007 in two farrow to finish farms in central Italy, where a total of 1400 sows were reared. The target population was divided into primiparous, pluriparous (1-5 parities) and old (>5 parities) sows. The faecal samples were collected at different stages of gestation: pre-partum (about 14 days before parturition), post-partum 1 (2 to 5 days after parturition), post-partum 2 (about 20 days after parturition) and gestation (24 to 31 days after parturition). On the farms, a cohort of 166 animals was selected, of which 102 underwent sampling for the whole period of the study; this reduction was due to the fact that some subjects died, were culled or did not result pregnant. To be better identified, the animals were individually ear tagged and divided into groups as reported in Table 1. To minimize the effect of seasonal variations in *Salmonella* excretion, the study was carried out in a period of one year, selecting batches of animals with different dates of delivery. Individual faecal samples, approximately 25 grams, were collected from the rectum of each animal four times, once for each of the phase of the reproductive cycle considered. In addition 182 environmental samples were collected from farrowing (#62) and gestation (#120) rooms, swabbing sponge bags (Solar Biologicals Inc) on surfaces (walls, floors, farrowing crates, nipple drinkers and feeders) before the introduction of the animals that had to be further sampled. Cleaning and disinfection were applied only in farrowing crates between different batches (all in- all out), while gestation rooms were managed on a continuous flow. All samples were stored in sterile containers and maintained at refrigeration temperature until processing. For *Salmonella* detection, 25 grams of faeces were processed according to the ISO 6579/2002 method. Environmental samples were soaked in 90 ml of buffered peptone water used as pre-enrichment and then processed according to the same method. Isolates of *Salmonella* from faecal or environmental positive samples were further serotyped according to the Kauffmann-White scheme.Moreover, the isolates from animals and the environment from the second farm, were further discriminated using PFGE, performed according to the Salmgene protocol (Peters, 2003). The DNA restriction was done using the XbaI enzyme. The images obtained have been evaluated using the BIONUMERICS software. For the statistical analysis of the results, the incidence density in days for each age group and the cumulative incidence (L.C. 95%) for the 4 phases of gestation were calculated.

Results

The results obtained from sows are summarised in Table 1. The calculated incidence density resulted 0.007 days for the primiparous, 0.005 for the pluriparous (1-5) and 0.001 for the old sows. The cumulative incidence resulted 0.6% (C. I. 95%, 0%-2%) in pre-partum, 2% (C.I. 95%, 0%-4%) in post-partum 1, 4% (C.I 95%, 1%-8%) in post-partum 2 and 27% (C.I. 95%, 18%-36%) in gestation. *Salmonella* Munchen and *Salmonella* Typhimurium were isolated on the first farm, while *Salmonella* serotype 4,5,12:i- and *Salmonella* Choleraesuis on the second farm. With regards to the environment, 5 out of 62 (8%) samples from the farrowing rooms and 28 out of 120 (23%) from gestation rooms resulted positive. On the first farm *S.* Munchen was isolated while, in the second farm, *Salmonella* serotype 4,5,12:i- and *S.* Typhimurium var. Copenhagen were found. The isolates of *Salmonella* serotype 4,5,12:i- were typed by PFGE and the profiles were analyzed using BIONUMERICS; different clones could not be discriminated within these isolates, since the genetic similarity ranged from 92 to 100%.

Discussion

The results of the present study showed that, on the whole, 6.6% of the faecal samples collected were positive for *Salmonella*. This feature confirms the results of similar investigations, reported in literature, where low percentages of faecal excretion, below 10%, were found in this category of animals (Kranker, 2003; Nollet, 2005). With regards to serotypes, the isolates, in particular *Salmonella* Typhimurium and *Salmonella* 4,5,12:i-, appear significant, since are some of the most frequently ones observed in swine at slaughter in EU (EFSA 2008).However, the overall percentage of contamination does not properly depict the epidemiological situation on the farms, since significant variations were observed in relation to age and physiological status of sows. In fact, the primiparous sows and the gestation phase showed significantly higher percentages of excretion in respect to the other groups. In respect to age, the rate of positive animals ranged from 2.0% in old sows to 10% in younger subjects; the statistical analysis confirmed this feature in terms of incidence density, from 0.007 days in the group of primaparous to 0.001 in old sows. Therefore, a more rapid spreading of infection is recorded in young animals with a decreasing trend related to the number of parities. These results confirm what was previously described by Nollet et al. (2005) and could be justified by a better immunological response in older animals. With regards to physiological status, the percentages ranged from 0.6% in prepartum to 26.5% in gestation and this trend was recorded within all the three groups (see Table 1). Also in this case, the statistical analysis allowed us to confirm the data, since the cumulative incidence is higher (27%) during gestation, compared to the other phases of the cycle. Therefore, the results demonstrate that gestation is the moment at major risk for excretion, confirming what described by other Authors (Nollet, 2005, Funk, 2001).Several factors were taken into account to justify this feature, such as the lower hygienic standard of the environment where the sows are kept during this stage, the stress linked to weaning and the major intake of water during lactation (Funk et al., 2001). In the present study, environmental sampling indicated higher contamination by *Salmonella* in the gestation boxes, before the introduction of animals that had to be further sampled. This was probably due to a different management of biosecurity in gestation rooms compared to farrowing rooms on both the farms, since all in-all out protocols were adopted for farrowing rooms, while gestation boxes were in a continuous flow. It is well known that the probability of infection depends on the quantity of *Salmonella* in the pigs' environment (Lurette 2008). In this work, higher environmental contamination was also related to a major number of excreting sows; this finding is supported by the comparison between the serotypes isolated from gestation boxes and those from sows introduced in the same environment. In fact, on farm A, *S.* Munchen was isolated from gestation boxes, and later, from the sows introduced in the same rooms. On farm B, instead, it was not possible to demonstrate this link, since the serotype, *S.* 4,5,12:i- was constantly isolated from animals and the environment on different sampling occasions and the molecular characterization, performed by PFGE, did not discriminate different clones within these isolates. On this farm, the presence of a single clone of *S.* 4,5,12:i- could be a consequence of the management of the herd, based on internal replacement of sows; therefore it could be hypothesized that this *Salmonella* strain had been circulating in this population for a long time.

Conclusion

The results suggest that the number of parities has a relation with *Salmonella* excretion; therefore a correct management of replacing gilts could be of help to reduce the contamination on breeding farms. In addition, biosecurity measures applied to gestation boxes have to be taken into consideration to reduce *Salmonella* spreading in closed pig herds.

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Table 1: Numbers and percentages of sows tested and positive for Salmonella

	Primiparous			Pluriparous (1-5)			Old (>5)			Total		
Time of gestation	Tested	Positive	%	Tested	Positive	%	Tested	Positive	%	Tested	Positive	%
Prepartum	45	1	2.2	52	0	0.0	65	0	0.0	162	1	0.6
Post-partum 1	42	3	7.1	51	0	0.0	61	0	0.0	154	3	1.9
Post-partum 2	38	1	2.6	51	4	7.8	50	1	2.0	138	6	4.3
Gestation	35	11	31.4	44	13	29.6	23	3	13.0	102	27	26.5
Total	160	16	10	198	17	8.6	198	4	2.0	556	37	6.6